Biochemistry and Biophysics Reports 9 (2017) 29–35

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Selective depletion of basophils ameliorates immunoglobulin E-mediated anaphylaxis

Takeshi Nakamura[a,b], Tomohiro Fukaya[a], Tomofumi Uto[a], Hideaki Takagi[a], Keiichi Arimura[a,c], Tetsuya Tono[b], Katsuaki Sato[a,*]

[a] Division of Immunology, Department of Infectious Diseases, Faculty of Medicine, University of Miyazaki, 5200 Kihara, Kiyotake, Miyazaki 889-1692, Japan
[b] Department of Otolaryngology, Head and Neck Surgery, Faculty of Medicine, University of Miyazaki, 5200 Kihara, Kiyotake, Miyazaki 889-1692, Japan
[c] Department of Oral and Maxillofacial Surgery, Faculty of Medicine, University of Miyazaki, 5200 Kihara, Kiyotake, Miyazaki 889-1692, Japan

ARTICLE INFO

Keywords:
Basophils
IgE
Type I hyperreactivity
Passive anaphylaxis
Intervention

ABSTRACT

Basophils, which are the rarest granulocytes, play crucial roles in protective immunity against parasites and development of allergic disorders. Although immunoglobulin (Ig)E-dependent responses via receptor for IgE (FcεRI) in basophils have been extensively studied, little is known about cell surface molecules that are selectively expressed on this cell subset to utilize the elimination *in vivo* through treatment with monoclonal antibody (mAb). Since CD200 receptor 3 (CD200R3) was exclusively expressed on basophils and mast cells (MCs) using a microarray screening, we have generated anti-CD200R3 mAb recognizing CD200R3A. In this study we examined the expression pattern of CD200R3A on leukocytes, and the influence of the elimination of basophils by anti-CD200R3A mAb on allergic responses. Flow cytometric analysis showed that CD200R3A was primarily expressed on basophils and MCs, but not on other leukocytes. Administration with anti-CD200R3A mAb led to the prominent specific depletion of tissue-resident and circulating basophils, but not MCs. Furthermore, *in vivo* depletion of basophils ameliorated IgE-mediated systemic and local anaphylaxis. Taken together, these findings suggest that CD200R3A is reliable cell surface marker for basophils *in vivo*, and targeting this unique molecule with mAb for the elimination of basophils may serve as a novel therapeutic strategy in ameliorating the allergic diseases.

1. Introduction-

Basophils are the least granulocytes, typically representing less than 1% of blood leukocytes, that have been implicated in the protective immunity against several pathogens, including parasites, and in the development of allergic disorders [1–3]. While basophils and mast cells (MCs) share some characteristics, including expression of the high-affinity receptor for immunoglobulin (Ig)E (Fcε receptor, FcεR) and release of allergy-inducing mediators such as histamine, basophils are distinct from MCs in several aspects, including development and anatomical localization, and life-span [1–3]. Although these apparent differences between basophils and MCs might suggest that they may have distinct roles *in vivo*, the literatures on the features of basophils have been limited owing to their rarity and similarities with MCs.

CD200 receptors (CD200Rs), members of the Ig superfamily, are composed of five distinct proteins, CD200R1, CD200R2, CD200R3, CD200R4, and CD200R5 in mice [4–6]. Although CD200R1 or CD200R2 has been implicated to function as high- or low-affinity receptor for CD200, the specific interaction between other CD200Rs and CD200 remains to be debated [4–7]. Whereas CD200R1 and CD200R2 is reportedly expressed on lymphoid cells and myeloid cells, including T cells, dendritic cells, MCs and basophils, CD200 have shown to be broadly expressed on a variety of cell types derived from hematopoietic and non-hematopoietic origins [4–7]. Previous studies have demonstrated that CD200R1 acts as an inhibitory receptor for subpopulations of macrophage lineages to control several immune responses [8,9]. Furthermore, the interaction between CD200 and CD200R1 reportedly led to the inhibition of degranulation and cytokine production in MCs and basophils upon FcεR stimulation [10,11]. Relative to CD200R1 and CD200R2, the characterization of

Abbreviations: BMMCs, bone marrow-derived mast cells; CD200R, CD200 receptor; DNP, 2,4-dinitrophenol; DNP-BSA, DNP-conjugated bovine serum albumin; FcεR, Fcε receptor; FcγR, Fcγ receptor; GFP, green fluorescent protein; Ig, Immunoglobulin; IL, Interleukin; IRES, internal ribosome entry site; ITAM, immunoreceptor tyrosine-based activation motif; mAb, monoclonal antibody; RBC, red blood cells; PE, Phycoerythrin; PSA, passive systemic anaphylaxis

* Corresponding author.
E-mail address: katsuaki_sato@med.miyazaki-u.ac.jp (K. Sato).

http://dx.doi.org/10.1016/j.bbrep.2016.11.001
Received 16 August 2016; Received in revised form 2 November 2016; Accepted 8 November 2016
Available online 10 November 2016

the other members of CD200R has not been well addressed.

Unlike other CD200Rs, CD200R3 appears to be mainly expressed on MCs and basophils with possible eight different splice versions (CD200R3A-H) in terms of variances of extracellular portion and cytoplasmic tails, and three different types of cytoplasmic tails might influence the association with an immunoreceptor tyrosine-based activation motif (ITAM)-bearing signaling adaptor molecules, including the Fcγ receptor (FcγR) chain as part of FcεR and both DAP10 and DAP12 [5,6,12,13]. It has been shown that cross-linking of CD200R3 with mAb (clones Ba91 and Ba103) induced degranulation in MCs and production of the cytokine in basophils *in vitro* [12]. However, the ligand(s) and the precise function(s) of CD200R3 remain still unclear.

Anaphylaxis is a rapid-onset, potentially life-threatening allergic reaction caused by the excessive release of allergic mediators after allergen exposure [14,15]. It has been well known that anaphylaxis is mainly caused by cross-linking of IgE-FcεR complexes on MCs [14,15]. Previous studies have suggested that basophils are one of the major mediators in the IgG-, but not IgE-mediated systemic anaphylaxis, distinctively from MCs, in mice when mAb to CD200R3 (clone Ba103) was used for specific elimination of basophils *in vivo* [16].

In this study, we examined the expression pattern of CD200R3A on leukocytes, and the influence of the depletion of CD200R3A$^+$ leukocytes on IgE-mediated experimental anaphylaxis, known as passive systemic anaphylaxis (PSA), in mice by utilizing anti-CD200R3A mAb (clone 6C4H2) [13]. In addition, we further addressed the role of CD200R3A in FcεRI-mediated activation of MCs.

2. Materials and methods

2.1. Mice

Female C57BL6/J mice were purchased from CLEA Japan (Tokyo, Japan). Animals were maintained in specific pathogen-free conditions in the animal facility at University of Miyazaki. All experimental procedures were performed with mice between 7 and 12 weeks of age in accordance with institutional guidelines of the Animal Experiment Review Board.

2.2. Cells

To prepare single-cell suspensions from spleen and lung, tissue samples were digested with collagenase type III (Worthington Biochemical, Lakewood, NJ) at 37 °C for 40 min, and were ground between glass slides. Splenocytes were treated with red blood cells (RBC) lysis buffer (Sigma-Aldrich, St. Louis, MO) before suspension. Bone marrow (BM) cells were flushed from the femurs and tibias. To prepare peritoneal exudate cells (PEC), the peritoneal cavity was lavaged with 10 ml of PBS using a 10 ml plastic syringe and 18-gauge needle. Single-cell suspensions were obtained by forcing through a 100-μm cell strainer (BD Biosciences, San Jose, CA). For preparation of transfectants expressing mock-green fluorescent protein (GFP) or CD200Rs-GFP, RBL2H3 cells were retrovirally transfected with pMX-internal ribosome entry site (IRES)-GFP vector (mock-GFP) as a control or pMX-CD200Rs-IRES-GFP vectors as described previously [13]. BM-derived MCs (BMMCs) were generated as previously described [12]. Briefly, BM cells were cultured in RPMI-1640 medium (Wako Pure Chemicals, Osaka, Japan) supplemented with recombinant murine interleukin (IL)-3 (4 ng/ml, Wako Pure Chemicals), 10% heat inactivated FBS, antibiotic-antimycotic (GIBCO BRL, Rockville, MD), 1 mM $_L$-glutamine, and 0.05 mM 2-mercaptoethanol for 4 weeks, and the cell population confirmed by flow cytometry in which CD49b$^+$FcεRIα$^+$ BMMCs represented 90% of the cells.

2.3. Flow cytometry

Cells were stained with fluorescein-conjugated mAbs to mouse CD3ε (145-2C11), CD4 (RM4-5), CD8α (53-6.7), CD11b (M1/70), CD11c (HL3), CD49b (HMα2), B220 (RA3-6B2), CD117 (2B8), isotype-matched control mAb (BD Biosciences), FcεRIα (MAR-1) (eBioscience, San Diego, CA). For CD200R3 staining, cells were stained with biotinylated mAb to CD200R3A (6C4H2), which was generated in our laboratory as described previously [13], plus streptavidin-PE (BD Biosciences). For staining with recombinant fusion proteins, cells were stained with Fc fragment of human IgG (huIgFc) or CD200-huIgFc [13] followed by anti-human IgG-PE (eBioscience). Fluorescence staining was analyzed with a FACSCalibur flow cytometer (BD Biosciences) and FlowJo software (Tree Star, Ashland, OR).

2.4. In vivo depletion of basophils

For the depletion of CD200R3A$^+$ leukocytes *in vivo*, mice were intravenously (i.v.) injected with anti-CD200R3A mAb (clone 6C4H2; 100 μg/mouse) or isotype-matched control Ig (100 μg/mouse; Sigma-Aldrich) through the tail vein one day before the antigenic sensitization.

2.5. Degranulation assay

Degranulation of MCs was measured by the release of β-hexosaminidase according to the previous report [12,17] with some modifications. Briefly, BMMCs were incubated overnight with 2,4-dinitrophenol (DNP)-specific IgE (clone SPE-7, 1 μg/ml; Sigma-Aldrich) in RPMI-1640 medium. After overnight incubation, the cells were stimulated with or without DNP-conjugated bovine serum albumin (DNP-BSA, 250 ng/ml; LSL, Tokyo, Japan) in the presence or absence of isotype-matched control Ig (10 μg/ml) or anti-CD200R3A mAb (clone 6C4H2; 10 μg/ml), or ionomycin (1 μm; Sigma-Aldrich) at 37 °C for 30 min in Tyrode's buffer. The supernatant was collected to determine the β-hexosaminidase activity and the cells were lysed with 1%-Triton X (Wako Pure Chemicals) in Tyrode's buffer. The supernatant and lysate were incubated with p-nitrophenyl *N*-acetyl β-gulcosaminide (Wako Pure Chemicals) in citrate buffer at 37 °C for an hour. The reaction was stopped by glycine and absorbance was measured with a spectrophotometer at 405 nm. The calculation of the release of β-hexosaminidase was determined by the following equation: (supernatant optical density)/(supernatant optical density plus cell lysate optical density) ×100.

2.6. Induction of passive systemic anaphylaxis

Induction and evaluation of passive systemic anaphylaxis were performed according to the previous report [16] with some modifications. In brief, mice were i.v. sensitized with DNP-specific IgE (50 μg in 300 μl PBS/mouse), and then the sensitized mice were injected i.v. with or without isotype-matched control Ig (100 μg/mouse) or anti-CD200R3A mAb (clone 6C4H2; 100 μg/mouse) 1 h after sensitization. Twenty-four hours after sensitization, the mice were i.v. challenged with DNP-BSA (50 μg in 300 μl PBS/mouse), and rectal temperature was measured by a digital thermometer (TD-300; Shibaura Electronics, Tokyo, Japan) every 5 min for 60 min.

2.7. Passive cutaneous anaphylaxis

Induction and evaluation of passive cutaneous anaphylaxis were performed as described previously [12] with some modifications. In brief, mice were intradermally (i.d.) sensitized with or without DNP-specific IgE (100 ng in 100 μl PBS/mouse) or control PBS in each ear, and then the sensitized mice were injected i.v. with or without isotype-matched control Ig (100 μg/mouse) or anti-CD200R3A mAb (clone 6C4H2; 100 μg/mouse) 1 h after sensitization. Twenty-four hours after sensitization, the mice were i.v. challenged with DNP-BSA (125 μg in 200 μl PBS/mouse) containing 0.8% Evan's blue dye (Sigma-Aldrich).

45 min after challenge, mice were sacrificed, and both ears were removed for measurement of vascular leakage of the dye. The dye was extracted from the ear by incubating *N, N*-dimethyl-formamide (Wako Pure Chemicals) at 55 °C for 3 h. The absorbance of the dye was measured with a spectrophotometer (iMark Microplate Reader; Bio-Rad, Hercules, CA) at 595 nm.

2.8. Statistical analysis

Data are the means ± s.d. from three to eight individual samples in a single experiment, and we performed at least four independent experiments. The statistical significance of differences between groups was analyzed using Student's *t*-test (two-tailed). P values < 0.05 or 0.01 were considered statistically significant.

3. Results

3.1. CD200R3A mainly expressed on basophils and MCs in vivo

As a microarray screening revealed that CD200R3 was predominantly expressed on basophils and MCs, we generated mAb against CD200R3A (clone 6C4H2) [13]. To examine the specific binding of anti-CD200R3A mAb (clone 6C4H2) to CD200R3A among CD200Rs, RBL2H3 cells expressing mock-GFP or CD200Rs-GFP were stained with anti-CD200R3A mAb (clone 6C4H2). Flow cytometry analysis showed that anti-CD200R3A mAb (clone 6C4H2) bound to RBL2H3 cells expressing CD200R3A, but not other transfectants (Fig. 1A). These results indicate that anti-CD200R3A mAb (clone 6C4H2) specifically recognizes CD200R3A among CD200R family molecules.

We next examined the binding of CD200 fusion protein, which consists of the extracellular domain of CD200 and huIgFc (CD200-huIgFc), to RBL2H3 cells expressing mock-GFP or CD200Rs-GFP (Fig. 1B). CD200-huIgFc bound to RBL2H3 cells expressing CD200R1 and CD200R2 to a lesser degree, indicating that CD200 is a ligand for CD200R1 and CD200R2 with high and low affinities. However, CD200-huIgFc did not bind to RBL2H3 cells expressing mock-GFP or other CD200Rs-GFP, suggesting that CD200 could not be a ligand for CD200R3 and CD200R4.

We addressed the expression pattern of CD200R3A on leukocytes. Flow cytometry with anti-CD200R3A mAb (clone 6C4H2) showed that the fractions of FcεRIα$^+$ cells and c-kit$^+$ cells expressed CD200R3A, whereas CD3$^+$CD4$^+$ T cells, CD3$^+$CD8$^+$ T cells, B220$^+$ B cells, CD11c^{high} dendritic cells, and CD11b$^+$CD11c$^-$CD49b$^-$ macrophages did not express this molecule (Fig. 1C). Indeed, the high expression of CD200R3A was only observed on FcεRIα$^+$CD49b$^+$ basophils and FcεRIα$^+$c-kit$^+$ MCs, but not other leukocytes (Fig. 1D). These results indicate that CD200R3A is predominantly expressed on basophils and MCs among leukocytes.

3.2. The administration of anti-CD200R3A mAb (clone 6C4H2) eliminates basophils in vivo

To clarify whether the administration with anti-CD200R3A mAb (clone 6C4H2) could deplete basophils and MCs *in vivo*, mice were systemically injected with this mAb, and we quantified the frequencies of FcεRIα$^+$CD49b$^+$ basophils and FcεRIα$^+$c-kit$^+$ MCs among leukocytes in spleen, lung, peripheral blood, and peritoneal cavity. A single injection with anti-CD200R3A mAb (clone 6C4H2) or anti-FcεRIα mAb (clone MAR-1) significantly ablated FcεRIα$^+$CD49b$^+$ basophils or CD200R3A$^+$CD49b$^+$ basophils in spleen, lung, and peripheral blood the next day after the injection (Fig. 2A-D). In contrast, there was no change in the proportion of FcεRIα$^+$c-kit$^+$ MCs and CD200R3A$^+$c-kit$^+$ MCs in peritoneal cavity after injection with anti-CD200R3A mAb (clone 6C4H2) or anti-FcεRIα mAb (clone MAR-1) (Fig. 2E-H). A maximal elimination of FcεRIα$^+$CD49b$^+$ basophils was achieved 24 h after the injection of anti-CD200R3A mAb (clone 6C4H2), but their proportion immediately rebounded thereafter, and had fully recovered by day 3 (Fig. 2I). These results indicate that administration with anti-CD200R3A mAb (clone 6C4H2) specifically eliminate basophils, but not MCs, *in vivo*.

3.3. Influence of anti-CD200R3A mAb (clone 6C4H2) on the activation of MCs

To address the effect of the ligation of CD200R3A by anti-CD200R3A mAb (clone 6C4H2) on the function of MCs, we performed a FcεR-mediated degranulation assay in BMMCs (Fig. 3A). In line with the published reports [12,17], cross-linking of FcεR with anti-DNP IgE mAb plus DNP-BSA induced the degranulation in BMMCs. On the other hand, treatment of BMMCs with anti-CD200R3A mAb (clone 6C4H2), but not control Ig, slightly induced the degranulation in BMMCs as compared with untreated BMMCs, while this treatment caused a further enhancement of their FcεR-mediated degranulation. These results indicate that anti-CD200R3A mAb (clone 6C4H2) act as an agonistic mAb to activate the function of MCs *in vitro*.

To further examine whether the administration with anti-CD200R3A mAb (clone 6C4H2) could induce the IgE-independent systemic anaphylaxis, mice were systemically injected with this mAb, and we monitored the body temperature of mice every 5 min for 60 min (Fig. 3B). However, the decline of the body temperature was not observed after the administration of anti-CD200R3 mAb (clone 6C4H2) as well as control Ig. These results indicate that anti-CD200R3A mAb (clone 6C4H2) does not elicit the IgE-independent systemic anaphylaxis *in vivo*.

3.4. Treatment with anti-CD200R3A mAb (clone 6C4H2) ameliorates IgE-mediated systemic and local anaphylaxis

To determine the protective effect of the administration with anti-CD200R3A mAb (clone 6C4H2) on IgE-mediated anaphylaxis *in vivo*, we evaluated the IgE-mediated systemic and local anaphylactic responses in the sensitized mice treated with this mAb. Mice that had been sensitized with DNP-specific IgE manifested not only systemic anaphylaxis marked by the decline of the body temperature but also local anaphylaxis as judged by increased vascular permeability and dye extravasation after the challenge with DNP-BSA (Fig. 4). In contrast, treatment of the sensitized mice with anti-CD200R3A mAb (clone 6C4H2), but not control Ig, exhibited the ameliorated systemic anaphylactic response (Fig. 4A). Furthermore, this treatment led to the inhibition of the enhanced vascular permeability measured by the leakage of dye as compared with untreated mice (Fig. 4B and C). Taken together, these results indicate that the elimination of basophils by the administration of anti-CD200R3A mAb (clone 6C4H2) protects from IgE-mediated systemic and local anaphylaxis *in vivo*.

4. Discussion

In this study, we demonstrate that CD200R3A is a specific cell surface molecule for basophils and MCs, and the treatment with anti-CD200R3A mAb is effective for protecting allergen-sensitized mice from the IgE-mediated systemic and local anaphylaxis mediated by the elimination of basophils *in vivo*.

While FcεRIα has been widely used for the detection of basophils and MCs in combination with other cell surface molecules (e.g., CD49b and c-kit), this cell surface molecule is also reportedly expressed on other cell types [18]. In consistence with the previous observations [12,13,16], we further confirmed that the expression of CD200R3A was restricted to basophils and MCs, but not other leukocytes, in various tissues. Therefore, CD200R3A is reliable cell surface marker for the detection of basophils and MCs *in vivo*. Accordingly, the detection of CD200R3A by anti-CD200R3A mAb (clone 6C4H2) might be useful for the clarification of their developmental process and anatomical locali-

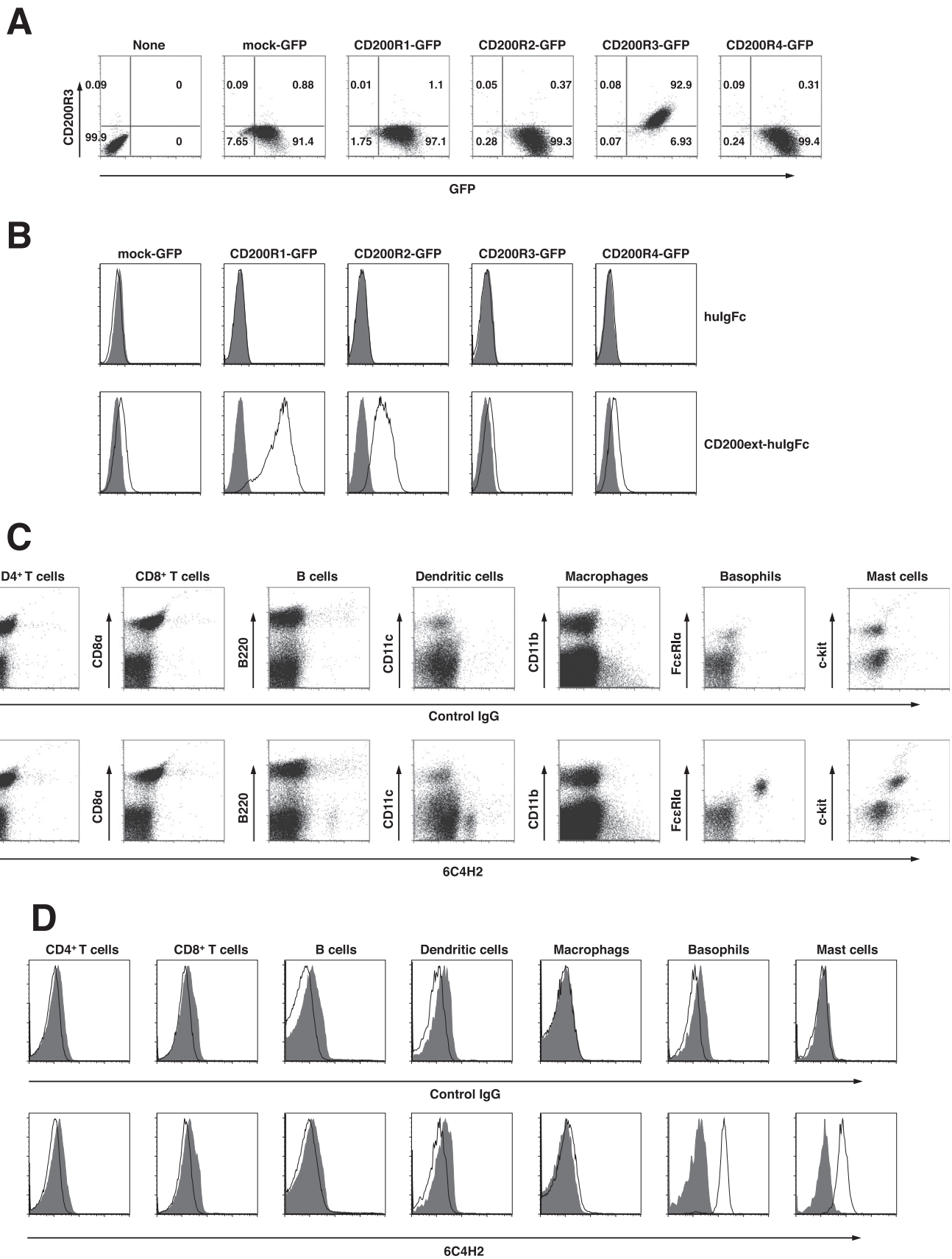
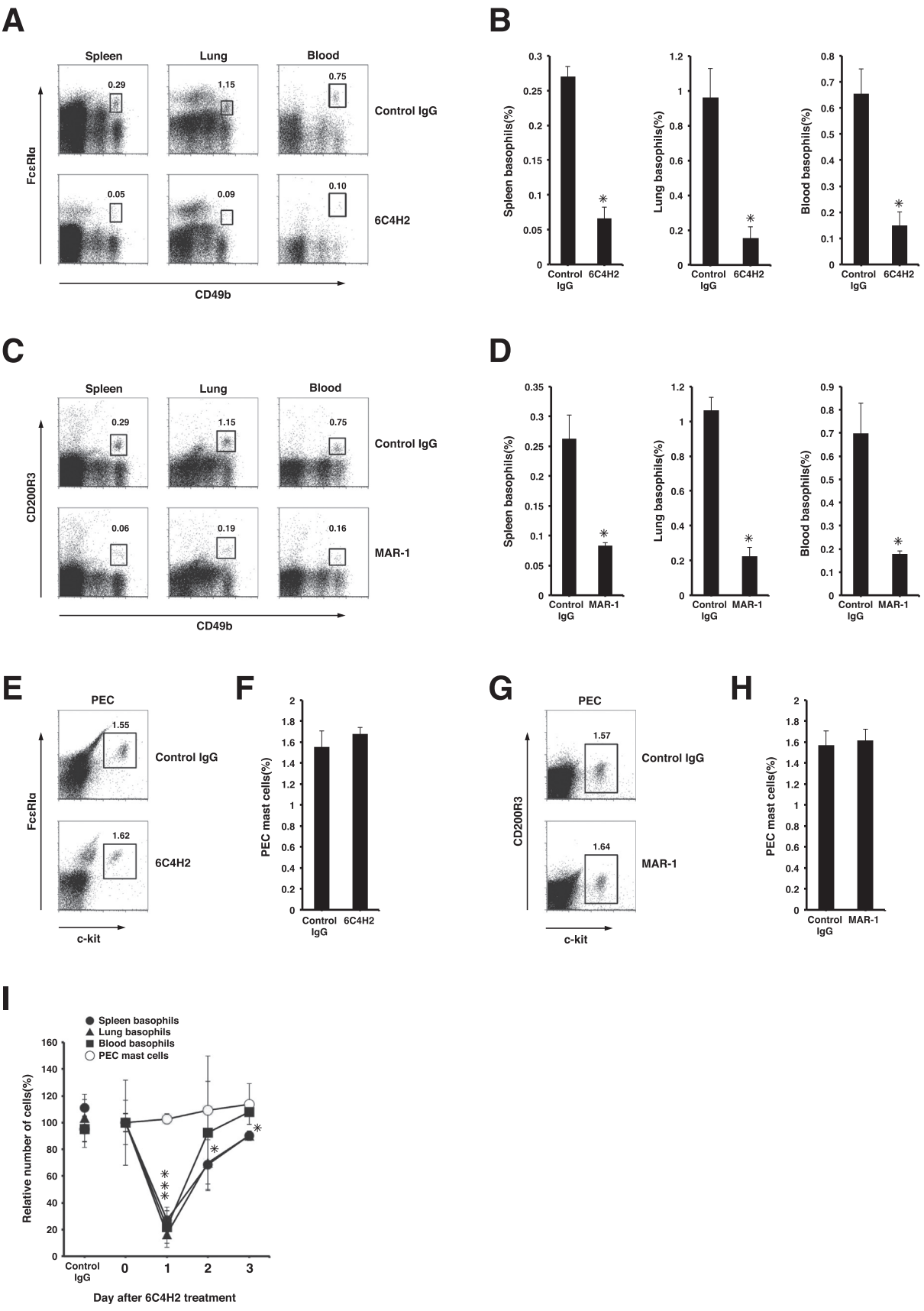


Fig. 1. Expression of CD200R3A on basophils and MCs. (**A**) Specific reactivity of mAb to CD200R3A (clone 6C4H2) against RBL2H3 cells expressing mock-GFP or CD200Rs-GFP was analyzed by flow cytometry, and data are represented by a dot plot. (**B**) Binding of CD200-huIgFc to RBL2H3 cells expressing mock-GFP and CD200Rs-GFP was analyzed by flow cytometry, and data are represented by a histogram. (**C**) The expression of CD200R3A and cell surface molecules on leukocytes was analyzed by flow cytometry with isotype-matched control Ig or anti-CD200R3A mAb (clone 6C4H2), and data are represented by a dot plot. (**D**) Cell surface expression of CD200R3A on $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells, $B220^+$ B cells, $CD11c^{high}$ dendritic cells, $CD11b^+CD11c^-CD49b^-$ macrophages, $Fc\varepsilon RI\alpha^+CD49b^+$ basophils, and $Fc\varepsilon RI\alpha^+$c-kit^+ MCs were analyzed by flow cytometry with isotype-matched control Ig or anti-CD200R3A mAb (clone 6C4H2), and data are represented by a histogram. Data are representative of four independent experiments with similar results.


A
Spleen
Lung
Blood
0.29
1.15
0.75
Control IgG
0.05
0.09
0.10
6C4H2
FcεRIα
CD49b
B
Spleen basophils(%)
Lung basophils(%)
Blood basophils(%)
Control IgG
6C4H2
C
CD200R3
0.29
1.15
0.75
Control IgG
0.06
0.19
0.16
MAR-1
CD49b
D
Spleen basophils(%)
Lung basophils(%)
Blood basophils(%)
Control IgG
MAR-1
E
PEC
1.55
Control IgG
1.62
6C4H2
FcεRIα
c-kit
F
PEC mast cells(%)
Control IgG
6C4H2
G
PEC
1.57
Control IgG
1.64
MAR-1
CD200R3
c-kit
H
PEC mast cells(%)
Control IgG
MAR-1
I
Spleen basophils
Lung basophils
Blood basophils
PEC mast cells
Relative number of cells(%)
Control IgG
0
1
2
3
Day after 6C4H2 treatment

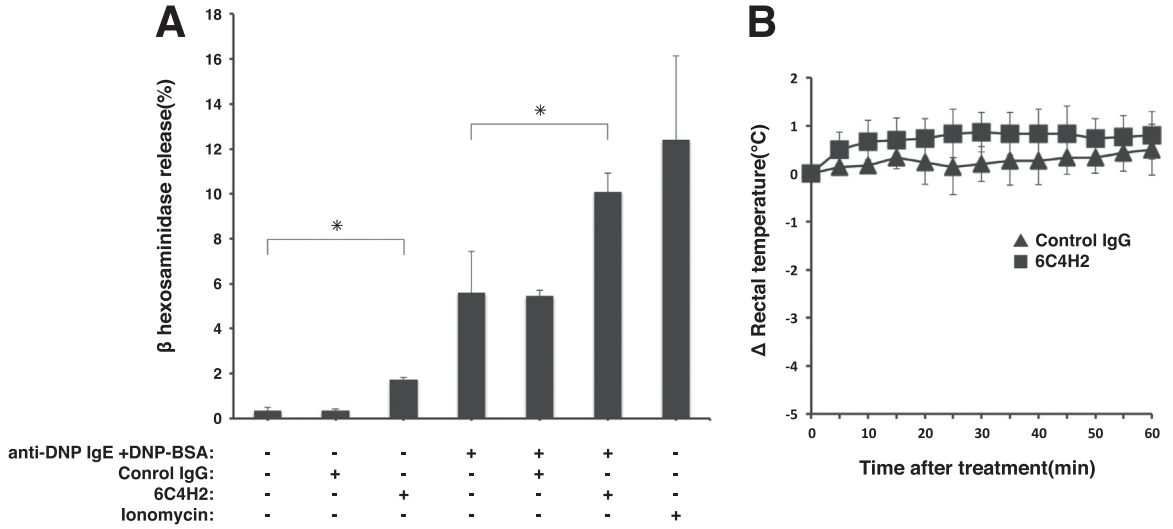


Fig. 3. Influence of the treatment with anti-CD200R3A mAb on IgE-dependent degranulation in MCs and induction of systemic anaphylaxis. (A) BMMCs were incubated with or without DNP-specific IgE, and then stimulated with DNP-BSA in the presence or absence of isotype-matched control Ig or anti-CD200R3A mAb (clone 6C4H2), or ionomycin as a positive control. β-hexosaminidase release was measured for the indication of MC degranulation. Data are the percentage of degranulated MCs among whole cells ± s.d. from four to eight individual samples in a single experiment. *P < 0.05 compared with untreated cells or among groups. (**B**) Mice were injected with control IgG (n=3) or anti-CD200R3A mAb (clone 6C4H2; n=3). Rectal temperature was monitored every 5 min for 60 min after the injection. Data are the mean ± s.d. from three individual samples in a single experiment. Data are

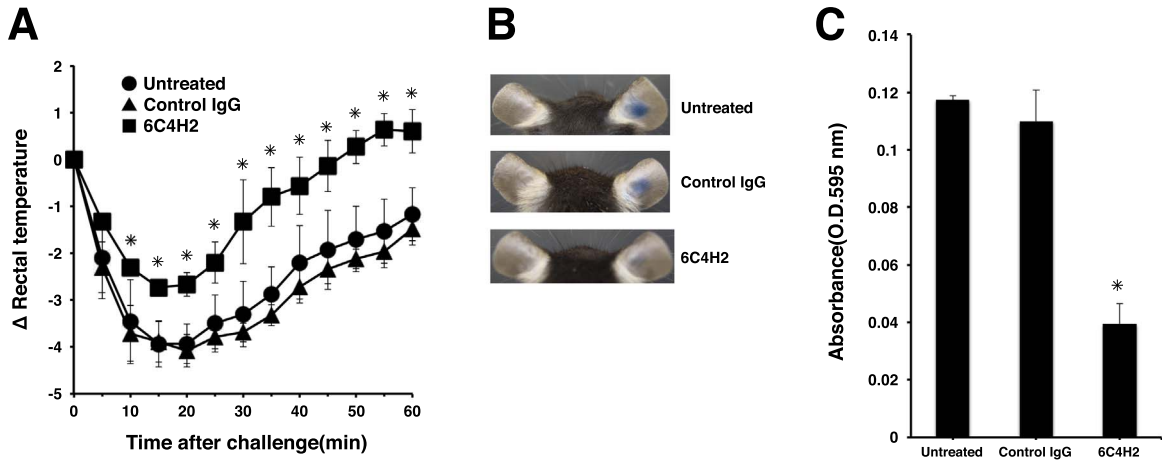


Fig. 4. Amelioration of IgE-mediated passive anaphylaxis by the administration of anti-CD200R3A mAb. (**A**) Mice that had been systemically sensitized with DNP-specific IgE were injected with or without isotype-matched control Ig or anti-CD200R3A mAb (clone 6C4H2; n=3) one hour after sensitization. Twenty-four hours after sensitization, mice were systemically challenged with DNP-BSA, and rectal temperature was monitored every 5 min for 60 min after antigenic challenge. Data are the mean ± s.d. from three individual samples in a single experiment. *P < 0.05 compared with untreated control. (**B** and **C**) Mice that had been sensitized with or without DNP-specific IgE in each ear were injected with or without isotype-matched control Ig or anti-CD200R3A mAb (clone 6C4H2; n=3) one hour after sensitization. Twenty-four hours after sensitization, mice were systemically challenged with DNP-BSA containing 0.8% Evan's blue dye. Forty five minutes after challenge, the photographs of the ears were taken (**B**) and the amount of vascular leakage was measured (**C**). Data are the mean ± s.d. from three individual samples in a single experiment. *P < 0.01 compared with untreated control. Data are representative of four independent experiments with similar results.

zation under physiological and pathological conditions.

Whereas one way to analyze FcεRIα$^+$CD49b$^+$ basophils and FcεRIα$^+$c-kit$^+$ MCs *in vivo* is to delete them through treatment with anti-FcεRIα mAb (clone MAR-1), this approach remains problematic because FcεRIα is also expressed on other leukocytes, resulting in the depletion of additional cell types. Similar to the previous reports with anti-CD200R3 mAb (clone Ba103) [16,19], the administration with anti-CD200R3A mAb (clone 6C4H2) led to the prominent *in vivo* depletion of CD200R3A$^+$FcεRIα$^+$CD49b$^+$ basophils, but not CD200R3A$^+$FcεRIα$^+$c-kit$^+$ MCs. The reason why the injection with anti-CD200R3 mAb (clones 6C4H2 andBa103) failed to deplete MCs remains unclear despite similar expression level of CD200R3 on basophils and MCs, this difference might be explained by their sensitivities to the Ab-dependent cellular cytotoxicity and FcγR–mediated phagocytosis as well as anatomical localization and life-span. Collectively, these finding suggest that the use of anti-CD200R3A mAb

Fig. 2. *In vivo* depletion of basophils by the administration of anti-CD200R3A mAb. (**A-I**) Mice were injected with control IgG (**A-I**), anti-CD200R3A mAb (clone 6C4H2; **A**, **B**, **E**, and **F**), or anti-FcεRIα mAb (clone MAR-1; **C**, **D**, **G**, and **H**), and spleen, lung, peripheral blood, PEC were obtained next day after the injection. The frequency of basophils or MCs was analyzed by flow cytometry. (**A**, **C**, **E**, and **G**) Data are represented by a dot plot, and numbers represent the proportion of FcεRIα$^+$CD49b$^+$ basophils (**A**) or CD200R3A$^+$CD49b$^+$ basophils (**C**) or FcεRIα$^+$c-kit$^+$ MCs (**E**) or CD200R3A$^+$c-kit$^+$ MCs (**G**) among leukocytes in each quadrant. (**B**, **D**, **F**, and **H**) Data are the mean percentage of FcεRIα$^+$CD49b$^+$ basophils (**B**) or CD200R3A$^+$CD49b$^+$ basophils (**D**) or FcεRIα$^+$c-kit$^+$ MCs (**F**) or CD200R3A$^+$c-kit$^+$ MCs (**H**) among leukocytes ± s.d. from three individual samples in a single experiment. *P < 0.05 compared with control IgG. (**I**) Mice were injected with control IgG or anti-CD200R3A mAb (clone 6C4H2), and spleen, lung, peripheral blood, PEC were obtained at the indicated days after the injection. The frequency of basophils or MCs was analyzed by flow cytometry. Data are the percentage of FcεRIα$^+$CD49b$^+$ basophils or FcεRIα$^+$c-kit$^+$ MCs among leukocytes ± s.d. from three individual samples in a single experiment. *P < 0.05 compared with control IgG. Data are representative of four independent experiments with similar results.

provides advantageous means to analyze *in vivo* unique function of basophils.

As anti-CD200R3 mAbs (clones Ba91 and Ba103) recognized both CD200R3A and CD200R3G, stimulation with these mAbs strongly activated basophils and MCs to degranulate and produce cytokine *in vitro* possibly mediated through the association of CD200R3 (isoforms A and G) with ITAM-bearing DAP12 and the activation of its downstream signaling cascades [12]. Furthermore, the injections with anti-CD200R3 mAbs (clones Ba91 and Ba103) caused IgE-independent systemic and local anaphylaxis in mice. Whereas the ligation of CD200R3A by anti-CD200R3A mAb (clone 6C4H2) slightly induced the degranulation in MCs, this treatment potentiated their FcεR-mediated activation. We also observed that the administration with anti-CD200R3A mAb (clone 6C4H2) did not enhance IgE-dependent systemic and local anaphylactic responses *in vivo* possibly owing to their ability to eliminate CD200R3$^+$ leukocytes, such as basophils and MCs, prior to their activation. The discrepancies on the functions of these CD200R3 mAbs might be due to their distinct recognition of CD200R3 isoforms exerting different function and epitopes or binding affinities. Given that basophils and MCs play crucial roles in the host defense against pathogens, such as parasites [1–3], CD200R3 might act as an activating receptor that recognizes certain pathogen-associated molecular patterns, independent of or synergistically with IgE-FcεR-mediated cellular activation. Further study will be needed to test this possibility.

Previous studies has shown that the depletion of basophils by anti-CD200R3 mAbs (clone Ba103) attenuated IgG-mediated systemic anaphylaxis, whereas this treatment had not effect on IgE-mediated systemic anaphylaxis in mice, suggesting that basophils are one of the major players in the IgG-mediated systemic anaphylaxis, but not IgE-mediated systemic anaphylaxis, while MCs are crucial for the development of IgE-mediated systemic anaphylaxis [14,16]. On the other hand, analysis of Mcpt8Cre-transgenic mice suggested that basophils were not required for passive IgE- or IgG1-mediated systemic anaphylaxis, whereas they were essential for IgE-mediated chronic allergic inflammation and protective immunity against secondary helminth infection [20]. We showed that the ablation of basophils, but not MCs, by anti-CD200R3A mAb (clone 6C4H2) ameliorated IgE-mediated systemic and local anaphylaxis *in vivo*. As anti-CD200R3A mAb (clone 6C4H2) and anti-CD200R3 mAbs (clone Ba103) [16,19] could exhibit a similar efficiency of depletion of basophils *in vivo*, the difference in CD200R3 isoforms with individual function and their epitopes recognized by these mAbs might explain the discrepancy on the mAb-mediated depletion of basophils on IgE-mediated systemic and local anaphylaxis *in vivo*. Therefore, the disparity among these observations regarding the role of basophils in IgE- or IgG1-mediated systemic anaphylaxis might be influenced by experimental conditions, such as mouse strains, experimental designs, and environmental factors. Taken together, our findings suggest that CD200R3A$^+$FcεRIα$^+$CD49b$^+$ basophils as well as MCs participate in the induction of IgE-mediated systemic and local anaphylaxis under certain conditions *in vivo*.

In conclusion, we described that CD200R3A are predominantly expressed on basophils and MCs, and it potentially functions as activating receptor on these cell type. Furthermore, the application with anti-CD200R3A mAb (clone 6C4H2) is effective for the amelioration of IgE-mediated anaphylaxis. Thus, targeting this unique human orthologue with mAb may constitute new therapy for basophil-mediated allergic disorders.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors thank all members of the animal facility at University of Miyazaki and Yumiko Sato for secretarial assistance. This work was supported by Grants-in-aid for Scientific Research (B) (K.S.) and for Young Scientists (B) (H.T. and T.F.) from the Ministry of Education, the Uehara Memorial Foundation (K.S. and H.T.), the Mochida Memorial Foundation for Medical and Pharmaceutical Research (K.S.), Takeda Science Foundation (H.T.), Kato Memorial Bioscience Foundation (H.T.), The NOVARTIS Foundation (Japan) for the Promotion of Science (H.T.), and Nagao Memorial Fund (H.T).

Appendix A. Transparency document

Transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2016.11.001.

References

[1] M.C. Siracusa, E.D. Wojno, D. Artis, Functional heterogeneity in the basophil cell lineage, Adv. Immunol. 115 (2012) 141–159.
[2] B. Pulendran, D. Artis, New paradigms in type 2 immunity, Science 337 (2012) 431–435..
[3] H. Karasuyama, Y. Yamanishi, Basophils have emerged as a key player in immunity, Curr. Opin. Immunol. 31 (2014) 1–7.
[4] R. Gorczynski, Z. Chen, Y. Kai, et al., CD200 is a ligand for all members of the CD200R family of immunoregulatory molecules, J. Immunol. 172 (2004) 7744–7749.
[5] D. Voehringer, D.B. Rosen, L.L. Lanier, et al., CD200 receptor family members represent novel DAP12-associated activating receptors on basophils and mast cells, J. Biol. Chem. 279 (2004) 54117–54123.
[6] G.J. Wright, H. Cherwinski, M. Foster-Cuevas, et al., Characterization of the CD200 receptor family in mice and humans and their interactions with CD200, J. Immunol. 171 (2003) 3034–3046.
[7] D. Hatherley, H.M. Cherwinski, M. Moshref, et al., Recombinant CD200 protein does not bind activating proteins closely related to CD200 receptor, J. Immunol. 175 (2005) 2469–2474.
[8] R.M. Hoek, S.R. Ruuls, C.A. Murphy, et al., Down-regulation of the macrophage lineage through interaction with OX2 (CD200), Science 290 (2000) 1768–1771.
[9] A.N. Barclay, G.J. Wright, G. Brooke, et al., CD200 and membrane protein interactions in the control of myeloid cells, Trends Immunol. 23 (2002) 285–290.
[10] S. Zhang, H. Cherwinski, J.D. Sedgwick, et al., Molecular mechanisms of CD200 inhibition of mast cell activation, J. Immunol. 173 (2004) 6786–6793.
[11] I. Shiratori, M. Yamaguchi, M. Suzukawa, et al., Down-regulation of basophil function by human CD200 and human herpesvirus-8 CD200, J. Immunol. 175 (2005) 4441–4449.
[12] T. Kojima, K. Obata, K. Mukai, et al., Mast cells and basophils are selectively activated in vitro and in vivo through CD200R3 in an IgE-independent manner, J. Immunol. 179 (2007) 7093–7100.
[13] K. Sato, K. Eizumi, T. Fukaya, et al., Naturally occurring regulatory dendritic cells regulate murine cutaneous chronic graft-versus-host disease, Blood 113 (2009) 4780–4789.
[14] K. Mukai, K. Obata, Y. Tsujimura, et al., New insights into the roles for basophils in acute and chronic allergy, Allergol. Int. 58 (2009) 11–19.
[15] S.R. Boden, A. Wesley Burks, Anaphylaxis: a history with emphasis on food allergy, Immunol. Rev. 242 (2011) 247–257.
[16] Y. Tsujimura, K. Obata, K. Mukai, et al., Basophils play a pivotal role in immunoglobulin-G-mediated but not immunoglobulin-E-mediated systemic anaphylaxis, Immunity 28 (2008) 581–589.
[17] K. Nishida, S. Yamasaki, Y. Ito, et al., FcεRI-mediated mast cell degranulation requires calcium-independent microtubule-dependent translocation of granules to the plasma membrane, J. Cell Biol. 170 (2005) 115–126.
[18] J.S. Shin, A.M. Greer, The role of FcεRI expressed in dendritic cells and monocytes, Cell. Mol. Life Sci. 72 (2015) 2349–2360.
[19] K. Obata, K. Mukai, Y. Tsujimura, et al., Basophils are essential initiators of a novel type of chronic allergic inflammation, Blood 110 (2007) 913–920.
[20] C. Ohnmacht, C. Schwartz, M. Panzer, et al., Basophils orchestrate chronic allergic dermatitis and protective immunity against helminths, Immunity 33 (2010) 364–374.